Supplementary information online

Supplementary methods:

Endocannabinoid measurements: Samples from individual mice were homogenized by using Pro 200 homogenizer (Pro Scientific Inc, Oxford, CT, USA) in 0.5 ml of an ice-cold solution of methanol/Tris buffer (50 mM, pH 8.0), 1:1, containing 7 ng of d4-anandamide, synthesized[1,2]. For extraction of endocannabinoids from nerve samples, nerves were pulverized in liquid nitrogen first and immediately extracted without initial homogenization. To each homogenate, 2 ml of ice-cold chloroform/methanol (1:1) and 0.5 ml of 50 mM Tris buffer, pH 8.0, was added. The homogenate was centrifuged at 4°C (500 × *g* for 2 min), the chloroform phase was recovered and transferred to a borosilicate tube, and the water phase was extracted two more times with ice-cold chloroform. The combined extract was evaporated to dryness at 32°C under a stream of nitrogen. The dried residue was reconstituted in 110 μl of chloroform, and 2 ml of ice-cold acetone was added. The precipitated proteins were removed by centrifugation (1,800 × *g*, 10 min), and the clear supernatant was removed and evaporated to dryness. The dry residues were reconstituted in 50 μl of ice-cold methanol, of which 35 μl was used for analysis by liquid chromatography/in line mass spectrometry, by using an Agilent 1100 series LC-MSD, equipped with a thermostated autosampler and column compartment. Liquid chromatographic separation of endocannabinoids was achieved by using a guard column (Discovery HS C18, 2 cm × 4.0 mm, 3 μm, 120A) and analytical column (Discovery HS C18, 7.5 cm × 4.6 mm, 3 μm) at 32°C with a mobile phase of methanol/water/acetic acid (85:15:0.1, vol/vol/vol) at a flow of 1 ml/min for 12 min followed by 8 min of methanol/acetic acid (100:0.1, vol/vol). The MSD (model LS) was set for atmospheric pressure chemical ionization, positive polarity, and selected ion monitoring to monitor ions *m/z* 348 for AEA, 352 for d4-AEA, and 379 for 2-arachidonoylglycerol (2-AG). The spray chamber settings were as follows: vaporizer, 400°C; gas temperature, 350°C; drying gas, 5.0 liters/min; and nitrogen was used as the nebulizing gas with a pressure of 60 psig. Calibration curves were produced by using synthetic anandamide and 2-AG (Cayman Chemical, Ann Arbor, MI). The amounts of AEA and 2-AG in the samples were determined by using inverse linear regression of standard curves. Values are expressed as fmol or pmol per paw.

***In situ* mRNA hybridization:** For generation of riboprobes, 1.5 kb-long CB_1-specific probes were generated from plasmid containing cDNA of CB_1[4]. 1.7 kb-long $GABA_{B(1)}$-specific probes were generated as previously described[5] and used as controls. *In situ* hybridisation using non-radioactive Dig-UTP-labeled antisense and sense probes was performed on cryostat sections of the spinal cord (20 μm) or DRG (16 μm) as described in details previously[5].

Immunohistochemistry: Mice were perfused with 0.1 M phosphate buffer saline and 4% paraformaldehyde (PFA) and the spinal cords and DRGs were isolated and post-fixed for up to 16 h in 4% PFA. Free-floating sections of mouse spinal cord (50 μm, vibratome) or frozen DRG sections (16 μm, cryostat) were shortly cooked in 10 mM Citrate buffer (18 mM Citric acid, 8.2 mM sodium citrate) for antigen retrieval and immunohistochemistry was performed using Rabbit anti-CB_1 antibody (Cayman), the corresponding biotinylated-secondary antibodies and standard reagent kits (Vectastain Elite ABC Kit, Vector laboratories, Burlingame, CA, USA) as described previously[6]. As markers to identify population of DRG neurons, an rabbit polyclonal antibody against substance-P, mouse monoclonal antibody against Neurofilament-200 and TRITC-conjugated Isolectin B_4 (IB_4-TRITC) were used. The anti-substance-P antibody and IB_4-TRIC were used to identify spinal nociceptive laminae. Brightfield and fluorescence images were taken using a cooled CCD camera (Leica, Bensheim, Germany) under similar illumination conditions.

Measurement of ERK1/2 phosphorylation and Fos expression in the dorsal root ganglia DRG and spinal cord *in vivo*: Mice in various treatment groups were subjected to hindpaw intraplantar injection with formalin (1%), as described in the main methods, killed and perfused transcardially with 4% paraformaldehyde at 1 h after formalin injection. Vibratome sections (50 μm) of the spinal cord or Cyrostat-sections (20 μm) of the L4/L5 DRG were immunostained with anti-phospho-ERK1/2 antibody (1:200) or anti-Fos antibody (Chemicon), as described above. **In some experiments, DRG sections were co-stained with anti-Fos and anti-CB_1 antibodies or anti-Fos and anti-Cre antibodies**. Immunoreactive cells in laminae I and II of the spinal dorsal horn were microscopically counted in 3-4 sections per mouse from 3 mice per treatment group as described by Ji *et.al*, 1999[7]. Similarly, the number of immunoreactive neurons per DRG section was counted and numbers were averaged over 10 sections per mouse and 3 mice per treatment group.

Receptor autoradiography on mouse spinal cord and DRG sections: Mice were killed using CO2 and the spinal cords segments L4-L6 and DRG were rapidly extracted and frozen on dry ice. Cryosections (16 μm) were co-incubated with 10 nM ^{3}H-CP-55940 (Perkin Elmer Life Science) and cold SR 144528, a CB_2 antagonist, for 2.5 hours at 37°C and processed for autoradiography as described in detail previously (Rubino et al., 2005). Autoradiograms were generated by the incubation of tritium-sensitive film (Hyperfilm-^{3}H, Amersham Pharmacia Biotech) with the sections for 7 days. The signal intensity was determined by measuring grey

levels using an image analysis system (Dual Scanner Artixscan 1800F & IMAGE-PRO-PLUS software) in the linear range after subtracting the background density.

Afferent recordings in skin-nerve preparation: Animals were killed by CO_2 inhalation, the saphenous nerve was dissected with the skin of the dorsal hind-paw attached and mounted in an organ bath "inside-up" to expose the chorium side. The preparation was superfused with an oxygen-saturated modified synthetic interstitial fluid solution containing (in mM) 108 NaCl, 3.48 KCl, 3.5 $MgSO_4$, 26 $NaHCO_3$, 1.7 NaH_2PO_4, 2.0 $CaCl_2$, 9.6 sodium gluconat, 5.5 glucose, 7.6 sucrose at temperature of 31 ± 1°C and pH 7.4 ± 0.05.

Recording technique: The saphenous nerve was pulled into a separate chamber of the organ bath and placed on a small mirror. Fine filaments were teased from the desheathed nerve and placed on a gold wire recording electrode. Action potentials in single sensory neurones were recorded extracellularly, amplified, filtered and visualised on oscilloscope.

Electrical stimulation: The nerve trunk was stimulated with square-waves pulses and the conduction velocity of the nerve fibres was computed from the distance between the stimulation and recording electrode and the latency of response to single pulse electrical stimulation. The fibres which conducted with < 2 m/s were considered as being unmyelinated (C) fibres.

Mechanical stimulation: Receptive fields of primary afferent fibres were identified by mechanical probing of the skin on the corium side with a glass rod. Once the receptive field was identified mechanical threshold of each unit was determined with a set of calibrated von Frey monofilaments with bending forces ranging from 1 – 362 mN. The strength of the finest filament which evoked at least 3 action potentials was defined as threshold.

Application of drugs: WIN 55,212-2 (Tocris Cookson, Bristol, UK) was dissolved in DMSO: Saline (1:1) solution and injected via intrathecal, intraplantar and intraperitoneal routes of administration. To enable intrathecal delivery at the level of lumbar spinal segments in mice, a polytetrafluoroethylene catheter (PTFE Sub-Lite Wall Tubing 0.05 mm ID x 0.15 mm OD; Braintree Scientific Inc., USA) was stereotactically inserted after hemilaminectomy at S1-S2 under isoflurane anesthesia. The intrathecal catheter was attached to a silicone tube, which was externalized. Mice were allowed to recover for 2 days after surgery and only animals showing complete lack of motor abnormalities were used for further experiments. The correct placement of the catheter was verified at the end of the experiment. WIN was administered intrathecally (10 g; 5 µl) after 17 h of intraplantar CFA injection using a microinjection syringe (Hamilton, Reno, USA). After injections, the catheter was sealed. Intraperitoneal injections of WIN 55,212-2 (1, 3 or 10 mg kg^{-1}) were performed in un-anesthetised mice in a total volume of 20 l.

For intraplantar injections, mice were shortly anesthetised by ether and WIN (10 g) was injected into the hind paw in a total volume of 20 l.

Nociceptive tests and mouse models of pain: Complete Freund's adjuvant (CFA, Sigma Aldrich) was injected unilaterally in the intraplantar surface of the hindpaw in mice (20 μl), whereas control mice were injected with 0.9% saline, as described in details previously[6]. Analysis of latency of paw withdrawal in response to noxious heat was done described in details[6] (Ugo Basile Inc.). Mechanical sensitivity was tested in the same cohort of animals via manual application of von Frey hairs to the plantar surface and via the use of an automated Dynamic aesthesiometer[6] (Ugo Basile Inc.). The 'spared nerve injury' (SNI) model for neuropathic pain following lesions of two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact has been described in details previously[8]. Cold allodynia following SNI was determined by counting the number of responses (flinching, licking, jumping, shaking) on a 5 °C cold plate during an observation period of 90 s, mechanical allodynia after SNI was measured as paw withdrawal latency to dynamic von Frey stimulation (Ugo Basile, maximum force 5 g, ramp 10 s)[9]. In some experiments, paw response to cold was determined by measuring the frequency of paw withdrawal in response to plantar application of acetone, which was applied once and the duration of withdrawal responses were recorded with a minimum value of 0.5 s and maximum of 20 s. Assessment of cannabinoid effects on thermal responses in the SNI model was done via a hot plate latency test. The hot plate was set at 50 °C and the latency to paw licking, paw withdrawal or jumping was measured. The intraplantar formalin test and capsaicin test were performed as described[6]. Acute pancreatitis was induced by intraperitoneal injection of caerulein[10] (50 μg kg^{-1} for each injection in saline; Bachem, Switzerland) 10 times at hourly intervals to mice. Frequency of abdominal nocifensive responses (licking of the abdomen, abdominal and/or whole body withdrawal) to graded punctuate abdominal pressure was analyzed using von Frey filaments (0.008 –0.6 g). Withdrawal frequency was calculated as the mean number of withdrawals out of 10 applications of the respective filament at 10 s intervals. The Rotarod test was used to analyse motor performance and the Ring catalepsy test[20] was performed to test WIN-induced immobility behavior.

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Supplementary figure legends:

Supplementary figure 1: Normal development of acute and pathological pain in SNS-Cre mice as compared to wild-type littermates (wt). (*a, b*) SNS-Cre mice demonstrate normal nocifensive responses following intraplantar injection of irritants (capsaicin and formalin). (*c, d*) Thresholds and magnitudes of responses to mechanical stimuli applied either as von Frey hairs (*c*) or via a Dynamic aesthesiometer (*d*) in basal (naïve) states or after intraplantar injection of Complete Freund's adjuvant (CFA) are comparable in SNS-Cre and wt mice. (*d*) Responses to von Frey, cold or heat stimuli prior to and after induction of spared nerve injury (SNI) are comparable in SNS-Cre and wt mice. $p > 0.05$ in all cases. All data points represent mean ± S.E. M.

Supplementary figure 2: Effects of WIN 55,212-2 (WIN), applied via intraplantar route of administration on inflammation-induced mechanical hypersensitivity in $CB_1^{-/-}$ mice and their wild-type littermates (wt). Intraplantar application of WIN (20 or 30 μg) significantly reduces CFA-induced mechanical hypersensitivity in wt mice (n = 5 for each dose), but not in $CB_1^{-/-}$ mice (n = 5 for each dose). * indicates $p < 0.05$ as compared to CFA-induced mechanical hyperalgesia before WIN administration, ANOVA, Fisher's test. All data points represent mean ± S.E. M.

Supplementary figure 3: Analysis of SNS-$CB_1^{-/-}$ mice and $CB_1^{fl/fl}$ mice in the spared nerve injury (SNI) model for neuropathic pain. (*a*) Responses to plantar application of acetone (cold stimulus) in SNS-$CB_1^{-/-}$ mice (n = 7) and $CB_1^{fl/fl}$ mice (n = 9). (*b*) Plantar response thresholds to von Frey hairs in SNS-$CB_1^{-/-}$ mice (n = 7) and $CB_1^{fl/fl}$ mice (n = 9; * $p = 0.02$, ANOVA, Fisher's test). All data points represent mean ± S.E. M.